TOXICITY OF SEVERAL NEW ORGANIC INSECTICIDES TO BODY LICE (PEDICULUS HUMANUS CORPORIS DeG.)

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Due to the length of the present paper it will appear in three parts[1], representing the three types of tests conducted. The first part will carry a review of the literature, but the references cited will appear in Part III.

All of the experimental phases of this work were conducted at the Orlando, Florida Laboratory, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. The writer wishes to acknowledge the assistance rendered by this laboratory in lending all the available facilities and personnel needed to complete these studies.

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PART I: BEAKER TESTS

The need for studies on the control of body lice, *Pediculus humanus corporis* deG. has been well brought out by historians who have dealt with the three important diseases transmitted by these insects—epidemic typhus, trench fever and relapsing fever.

The ravages of epidemic typhus and the role that lice have played in this disease has been dramatically portrayed by Zinsser (1944). This author pointed out that louse-borne typhus has been one of the epidemic diseases most feared by the military strategist and that battles and indeed even wars have been decided by it. One of the most noteworthy examples of the importance of lice and the diseases they carry is that of Napoleon's retreat from Russia even after the invasion of Moscow. The plight of his retreating soldiers is history, and Napoleon, one of the greatest generals, was defeated—not by the Russians—but by the louse and the death dealing organism it transmits.

Even at the outset of World War II epidemic typhus was one of the most dreaded diseases. Leading medical men and their scientific associates made careful plans for combatting this fatal disease. The development of DDT during the war and the accomplishment of typhus control in Italy, and other European countries, as well as Japan, will stand out in history as one of the high lights of scientific accomplishments.

One, however, should not forget that the control of louse-borne typhus might not have been possible had it not been for the brilliant work of several biologists, some of whom lost their lives to epidemic typhus.

Bayne-Jones (1948) states that the achievement at Naples, Italy, in 1943–1944 in the control of epidemic typhus stems from two fundamental discoveries made in 1909; one was the proof by Charles Nicolle (Nicolle 1909; Nicolle et al 1909) that

body lice transmit typhus fever from man to man, the other by Ricketts (Ricketts, 1909, Ricketts and Wider, 1910, 1910a, 1910b, and 1910c), who discovered the causative organism of typhus, *Rickettsia prowazeki.* The discovery by Nicolle led Zinsser (1943–1944) to state that: "The strategic initiative passed into the hands of men, with the discovery in 1909 by Charles Nicolle, of the louse transmission of typhus from man to man. The victim was in a position to organize a rationally planned and strategically sound defense against his historic enemy". Further proof of the discovery was given by Strong et al (1920) and Walbach et al (1922) who confirmed that in the absence of lice, typhus is not contagious. As pointed out by Bayne-Jones (1948) typhus is associated with louse feces, and the occasional transmission of typhus by breathing dust infected with louse feces is still an association of man with lice. One may go still further and state that healthy lice cannot transmit the typhus organism and this knowledge led to the isolation of thousands of typhus cases in both Europe and Japan during World War II (Bayne-Jones, 1948; Gordon 1948; Scoville 1948; and Blanton 1951).

The other two diseases, trench fever and relapsing fever, which are transmitted by body lice are of much less importance than epidemic typhus. They, of course, are capable of causing great morbidity.

In this paper, data are presented on the relative toxicity of several organic insecticides to adult body lice, to determine if some would be even more useful than DDT louse powders.

At the time this research was being conducted it was thought that this information would be needed in case DDT resistance develops in louse populations, and subsequently resistance has been encountered in Japan and Korea, thus making necessary the use of alternate insecticides. Also the most probable large scale use of insecticides for louse control would come during and immediately following major national disasters. Substitute material should, therefore, be known to better insure an adequate supply of an effective chemical.

The experimental work for these papers was conducted at the Orlando, Florida, Laboratory of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. The availa-

bility of a large louse colony and all chemicals needed made this location the most logical choice.

The chemicals used in these papers are listed according to the Chemical Abstracts System. For convenience they are arranged alphabetically. Common names, when known, are given after the name in parentheses. The thirteen chemicals used more often are as follows:

Camphene, chorinated (toxaphene)
Cyclohexane, 1,2,3,4,5,6, 95–100% gamma isomer (lindane)
Cyclohexane, 1,2,3,4,5,6-hexachloro-(10–12% gamma isomer)
1,4,5,8-Dimethanonaphthalene, 1,2,3,4,10,10-hexachloro-6,7-epoxy-l. 4,4a,5,6,7,8,8a-octahydro-(dieldrin)
1,4,5,8-Dimethanonaphthalene, 1,2,3,4,10,10-hexachloro-1,4,4a, 5,8,8a-hexahydro-(aldrin)
Ethane,1,1,1-trichloro-2,2-bis(*p* chorophenyl) (DDT)
Isobornyl sulfone, chlorinated, 70.5% Cl.
4,7-Methanoindene, 1(or 3a), 4,5,6,7,8-heptachlor-3a,4,7,7a-tetrahydro-(heptachlor)
4,7-Methanoindene,1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-(chlordane)
Pinene, chlorinated, 68.5% Cl.
Pinene, chlorinated, 68.8% Cl.
Sulfone, isobornyl phenyl, chorinated, 66.1% Cl
Thiophosphoric acid, O,O-diethyl-O-(p-nitrophenyl)ester (parathion)

HISTORY OF BODY LOUSE CONTROL SUBSEQUENT TO THE BEGINNING OF WORLD WAR II

A brief review of the literature is presented which brings together some of the more important titles on the subject of lice and their control that have been published since Grinnell and Hawes (1943). This publication covered the literature from 1758 through 1942.

During World War II modern methods of louse control replaced the cumbersome methods previously used. Methyl bromide was substituted for the steam method for treating clothing at most ports where German and Italian prisoners of war were being deloused. (Latta and Yeomans 1943, Latta 1944, Latta et

al 1946, Stone and LaHue 1946, Richardson 1947, Blanton 1951). Individual bags were developed to fumigate soldiers' clothing, but these were seldom used A glass ampule holding 20 ml. of methyl bromide was developed for use with this bag. Although methyl bromide fumigation was an improvement over steam, the method still had many faults, being cumbersome, slow, and because of the extreme toxicity of the gas, dangerous for operating personnel (Houghton, 1946). Fumigation chambers were used at ports and to a limited extent in the field.

Fumigation with hydrocyanic acid gas was also used to some extent for disinfecting clothing and buildings (MacKenzie, 1941; Sherrard, 1942; David, 1944). Hot and cold air was used in some areas for control of lice in clothes (Busvine, 1944). In Germany one worker recommended placing clothing on ant nests (Hose, 1942).

While these methods were being used, laboratories were conducting research on the development of chemicals for louse control. Out of this work MYL and DDT louse powders and other DDT formulations were developed in the United States, AL63 in England and Lauseto Neu in Germany. The first official United States louse powder developed contained 0.2% pyrethrins, 2% dinitroanisol, 2% n-isobutylundecyleneamid and 1% phenol S(isopropyl cresols) in pyrophyllite. This product was known as MYL (Bushland, McAllister et al, 1944). MYL was later replaced by DDT, a product synthesized as early as 1874, but which lay dormant for many years without further development (Zeidler, O., 1874).

Bayne-Jones (1948) has reviewed the introduction of this material to the United States. Knipling (1948) states that: "In November 1942 a prepared insecticide called 'Gesarol Dust Insecticide' was received from the J. R. Geigy Company of New York, a subsidiary of the Geigy firm in Switzerland, where it was developed through the important research of Paul Mueller and his associates. A sample of the insecticide was obtained through the Division of Insecticide Investigations of the Bureau of Entomology and Plant Quarantine. This prepared insecticide contained as the active principle the material now known as DDT. . . ."

Other developments were being made by other laboratories on chemicals for lice control. Slade (1945) published on the new British insecticide, benzene hexachloride, and Gomez Ozamiz (1945) mentions using it in Spain for the control of various insects including lice.

A good deal of work was done in Russia with dixanthogen ($C_6H_{12}O_2S_4$) for louse control. This material was usually referred to as "K" or "SK" (Gorkina, 1942; Ivanova, 1942; Soboleva, 1942, 1942a).

Most of the chemicals mentioned above were developed for external use as powders. Jones et al (1945) published on impregnation of clothing and Jones and Fluno (1947) on xylene emulsions of DDT for louse control. Musgrave (1946) and Goodall et al (1946) also published on impregnation of clothing. Busvine (1945) describes a belt which was impregnated with "lasting insecticides" and worn for louse protection.

Another development during the war which should be continued was the protection of animals through internal medication, Knipling et al (1948). It is possible that a chemical could be found which would be compatible toxicologically, to the human and at the same time give protection against insects. This field of research needs a great deal more investigation yet is not within the scope of these studies.

All of these developments aided in louse control but the developments of louse powder at the Orlando Laboratory contributed more to the control of epidemic typhus than any other single development. In experimental and practical tests MYL proved to be very effective, (Bushland, McAllister et al, 1944; Bushland Schecter et al, 1944; Davis and Wheeler, 1944). This material was found to be completely effective for breaking an epidemic of typhus in a Mexican village (Davis et al, 1944).

MYL, however, was soon replaced with DDT which had been proven experimentally to be a very effective louse powder with long residual action (Bushland et al., 1944, 1945; Knipling, 1946; Stone, 1946). From these early discoveries the magic of DDT became the byword of at least all preventive medicine officers, accounting for the large number of publications on the control of insects which carry human disease. A review of this work is be-

yond the scope of this paper, but mention should be made of some of the outstanding work of a few of the individuals who contributed so much to the control of lice. As chief of the United States of America Typhus Commission, Stanhope Bayne-Jones (1943, 1948) contributed enormously. Numerous papers have appeared giving the excellent results of DDT for the control of lice (Bushland, 1948; Bushland et al., 1944; Eddy, 1944, 1946, 1948; Eddy and Carson, 1946, 1947, 1948; Eddy, Carson and Bushland, 1947; Knipling, 1946, 1948; Knipling et al., 1944; Buxton, 1945; Stowman, 1945; Simmons, 1945; Soper, 1945; Stone, W. S., 1946; Wheeler, 1946; Busvine, 1946, 1947; Canadel Vidal, 1946; Chalke, 1946, 1946a; Crauford-Benson, 1946; Goodall et al., 1946; Gordan, 1948; Scoville, 1948; Sergent, 1944; and Blanton, 1951, 1951a.

DDT powders were used at a strength of 10% toxicant and 90% pyrophyllite. The powder was furnished in 2 ounce cans for the individual soldier's use and also in bulk for group delousing.

According to Bayne-Jones (1948), Wheeler was the first to use dust-guns for applications of louse powders to humans without having the infested person disrobe. This method later proved to be a fast, efficient method for delousing large numbers of individuals. A power duster was later developed which was capable of dusting 600 persons per hour.

With the extraordinary results obtained with DDT for the control of lice, it is easy to overlook the phases of typhus control which are likely as important as the development of DDT for this particular disease. Typhus vaccine developed by the Cox method (Cox and Bell, 1940) was important in protecting soldiers from death. During the war there were 64 known cases of epidemic typhus in the US Army and although the symptoms were usually mild, they were sometimes severe. Nevertheless, there was a potential death with each case and without vaccine protection some deaths certainly would have occurred. The author was present in Osaka, Japan, for almost the duration of the epidemic, the largest experienced during the war (Scoville, 1948, Blanton, 1951a). Little has been published on that epidemic, but the

death rate of the Japanese natives subsided several percent after the use of the vaccine.

In the experimental studies which were responsible for the development of louse control measures thousands of lice were needed daily. Mention should be made of the work of Culpepper (1944, 1946, 1946a, 1948) who worked out the technique for rearing lice on rabbits.

DESCRIPTION OF METHODS AND RESULTS OF TESTS

Three types of tests were employed in these studies: (1) beaker test, (2) dust test, and (3) fumigation test. The beaker tests were designed primarily to screen large numbers of chemicals, but the method was expanded to test the limits of dilution and the lasting properties of the chemical. The dust tests were used to test the practicability of any promising louse toxicant found in the beaker tests. The promising chemicals were tested at different concentrations to determine the lowest effective dilution. Since many of the compounds were found to be effective at extremely low dilutions the concentrations of toxicants are given in parts per million instead of in terms of percentages.

Beaker Tests

Methods used for screening compounds:—The beaker test method developed by Bushland et al. (1944b) and also Eddy and Carson (1946) was used in the screening tests for initial kill and lasting qualities of new compounds. In these tests circular woolen cloth pads $1\frac{1}{2}$ inches in diameter were dipped in 1% (10,000 p.p.m) solutions of the test materials, and then were suspended on pins to dry at room temperature. When thoroughly dry, individual pads were placed in 5 ml. beakers and ten young adult lice were added for each test. Most of the compounds were applied to the pads from acetone solutions. Those that were not soluble in this material were dissolved in other suitable volatile solvents such as chloroform, benzene, alcohol or water.

The initial kill of the compound was based on the mortality after 24 hours' exposure. The lasting qualities of the chemical were determined by adding ten new lice daily until normal lice

survived. All materials which gave complete kill for ten days were subjected to additional tests.

Screening large series of chemical compounds to test effectiveness against adult lice:—During the war thousands of chemicals were tested as toxicants against the body louse. Up until September 1947 approximately 7,000 materials had been tested, Eddy, Carson, and Bushland (1947). Since that time over 3,000 new materials have been tested. As mentioned above, those chemicals which were effective for more than ten days in beaker tests were tested further.

Minimum concentration of compounds which were effective for 31 days or more:—The chemicals discussed in these papers were effective for 31 or more days in beaker tests. Dilution tests were made to eliminate the poorer lousicides from further consideration. In the preliminary tests the chemicals were diluted to 1,000, 100 and 50 parts per million. In each test ten lice were used.

A series of 106 chemicals had given 100% louse mortality for 31 days at 10,000 p.p.m. (1%). Further tests with these chemicals yielded the following results: at dilution of 1000 p.p.m., 40 compounds gave 100% kill; at 100 p.p.m. only 14 gave 100% kill; and at 50 p.p.m. only 8 gave 100% kill. DDT gave 100% kill only at 1000 p.p.m., 70% kill at 100 and 30% kill at 50 p.p.m. DDT, the standard lousicide was selected as the chemical with which to compare all other chemicals.

Prior to some of the preceding tests, preliminary tests were conducted with some new chemicals including chlordane and dieldrin. These showed remarkable toxicity to lice as well as to other insects.

The results of further dilutions of these chemicals are presented in Table I. Three additional dilutions, 100, 50 and 25 parts per million were not included in the table. At these dilutions dieldrin and chlordane gave 100% kill, compared to DDT which gave 100, 50 and 30% kills at the respective dilutions. Dieldrin gave 100% kill at a dilution of 6.25 parts per million in all seven tests and 100% kill in three of the seven tests at a dilution of 3.125 p.p.m. In six tests at a dilution of 0.1 p.p.m., 100% kill was had in four tests. Chlordane gave 100% kill at 6.25 p.p.m., 88% kill at 3.125

p.p.m., and 76% at 0.1 p.p.m. DDT had lost most of its toxicity at 12.5 p.p.m. giving only 10% kill.

TABLE 1. AVERAGE PERCENT MORTALITY OF ADULT LICE WITH VARIOUS CONCENTRATIONS OF DIELDRIN, CHLORDANE, AND DDT. EACH TEST WITH 10 LICE

Chemical	Parts Per Million				
	12.5	6.25	3.125	0.1	0.05
Dieldrin	100	100	88.6	71.6	0
Chlordane	100	100	88	76	
DDT	10				

In 10 check lots of 10 lice each there were 2 dead lice or 2% mortality.

Two samples of chlordane were tried at dilutions of 50, 25, 10, 5 and 2.5 p.p.m. All tests at 50 and 25 p.p.m. resulted in 100% kill in both samples. Over 90% was had at 10 p.p.m. and even at 5 p.p.m. over 50% kill resulted. At 2.5 p.p.m. there was almost 50% kill with both samples. In four checks of ten lice each there was one dead louse or 2.5% mortality.

Thirteen of the better chemicals were selected from the preliminary tests for trials at lower concentrations. These concentrations ranged from 100 to 1 part per million. A minimum of two tests were made, but in some cases twelve tests were made on different days.

The results of these dilution tests are presented in Table 2. At concentrations of 100 parts per million, DDT which gave an average kill of 88% in four tests was definitely inferior to all other chemicals. At 50 parts per million, DDT with an average of 63% mortality was superior both to toxaphene with an average of 48% for four tests and chlorinated pinene (68.5% Cl.) with an average of 53% mortality. It was slightly inferior to two other materials, chlorinated isobornyl phenyl sulfone (66.1% Cl.) and chlorinated pinene (68.8% Cl.) with 65 and 68% respective mortality. At a concentration of 25 parts per million DDT gave 15% kill which was superior to three other chemicals.

The next concentration, 12.5 parts per million completely eliminated DDT, benzene hexachloride (12% gamma), toxaphene, chlorinated isobornyl sulfone (63.3% Cl.), chlorinated isobornyl

phenyl sulfone (66.1% Cl.), chlorinated pinene 68.8% Cl.), and chlorinated pinene (68.5% Cl.). There were still four chemicals, parathion, heptachlor, aldrin and dieldrin, which gave 100% kill at the 12.5 p.p.m. concentration.

At concentrations of 6.25 parts per million three chemicals, heptachlor, aldrin and dieldrin, still gave 100% kill. At the next dilution, 3.125 parts per million, only one chemical, aldrin gave 100% kill in all tests, however, heptachlor and dieldrin gave respective average kills of 90 and 85%.

TABLE 2. AVERAGE PERCENT OF LOUSE MORTALITY AFTER 24 HOURS EXPOSURE ON CLOTH PATCHES TREATED WITH LOW CONCENTRATIONS OF TOXICANTS. FOUR TO SIX TESTS OF 10 LICE EACH.

Chemical	Number of Tests	Concentration in Parts Per Million						
		100	50	25	12.5	6.25	3.125	1
Aldrin	6	100	100	100	100	100	100	0
Heptachlor	12	100	100	100	100	100	90	5
Dieldrin	4	100	100	100	100	100	85	0
Parathion	4	100	100	100	100	68	10	0
Lindane	6	100	100	100	93	82	52	0
Chlordane	10	100	100	100	86	29	8	0
Technical benzene hexachloride (12% gamma)	2	100	100	70	30	0	0	
Isobornyl sulfone, chlorinated (63.3% Cl.)	4	100	98	13	3	0	0	
Pinene, chlorinated (68.8% Cl.)	4	98	68	40	0	0	0	
Sulfone, isobornyl phenyl, chlorinated (66.1% Cl.)	4	100	65	30	3	0	0	
Pinene, chlorinated (68.5% Cl.)	4	100	53	5	0	8	0	
Toxaphene	4	100	48	8	3	0	5	
DDT	4	88	63	15	3	5	0	

There were 2 dead lice in 24 checks of 10 lice each or a mortality of 0.8%.

Durability of the better chemicals found in the initial screening: New chemicals were found which were a great deal more toxic to lice than the older chemicals including DDT. Many of these could be diluted more than DDT and still maintain a high level of louse control. Thirteen of the best chemicals were chosen for dilution-durability studies. Cloth patches were treated with

each chemical in five concentrations, 50, 25, 12.5, 6.25, and 3.125 parts per million. After 24 hours the mortality was checked and new lice were placed on the patches that were still effective. This process was repeated daily until the chemical had definitely failed.

Concentrations of 50 parts per million DDT gave a 50% kill for one day and no kill thereafter. The remaining 12 chemicals were superior to DDT at 50 parts per million as they were effective for more than one day. The outstanding chemicals were parathion and dieldrin. With concentrations as low as 12.5 parts per million parathion gave 100% kill through the eighth day and dieldrin through the fifth day.

(*To Be Continued*)